International Acceptance of the Nonradioactive LLNA: DA for Evaluating Allergic Contact Dermatitis Hazards

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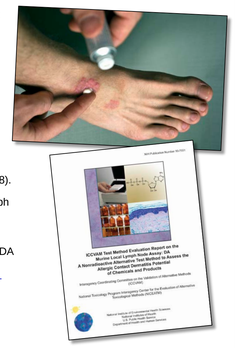
Introduction

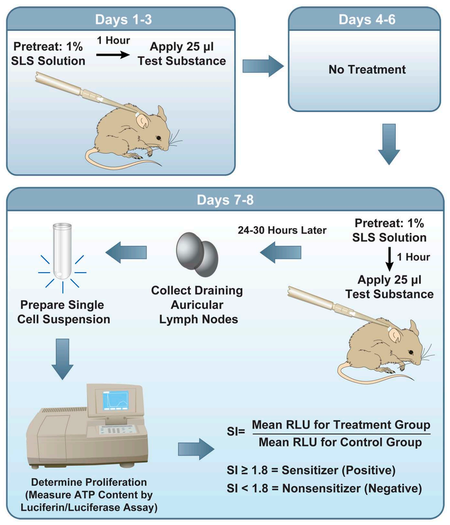
- The murine local lymph node assay (LLNA) is a test method for assessing the potential of substances to cause allergic contact dermatitis (ACD). ACD is an allergic skin reaction characterized by redness, swelling, and itching that can result from repeated contact with a sensitizing substance.
- In response to a nomination by the U.S. Consumer Product Safety Commission in 2007, NICEATM evaluated the nonradioactive LLNA: DA (**Figure 1**) to assess the ACD hazard potential of substances.
- Daicel Chemical Industries, Ltd., developed the LLNA: DA (Yamashita et al. 2005; Idehara et al. 2008).
  - Measures ATP content in draining auricular lymph nodes as an estimate of cell number for the assessment of lymph node cell proliferation.
- ICCVAM published recommendations on the LLNA: DA in a test method evaluation report (available at: http://iccvam.niehs.nih.gov/methods/immunotox/llna-DA/TMER.htm).



LLNA: DA Test Method Protocol

- The LLNA: DA protocol (**Figure 1**) incorporates all aspects of the traditional LLNA protocol except for those procedures unique to the conduct of the LLNA: DA:
  - Pretreatment with 1% sodium lauryl sulfate prior to test substance application
  - One additional day of test substance application after 3 days of no treatment
  - Assessment of proliferation by measuring intracellular ATP levels within lymph node cells instead of ^{3}H-thymidine incorporation
- The reduced LLNA: DA (rLLNA: DA) should be considered and used to determine the ACD hazard potential of chemicals and products in testing situations where dose-response information is not required or negative results are anticipated.
  - Like the reduced LLNA (Kimber et al. 2006; ESAC 2007; ICCVAM 2009), the rLLNA: DA protocol uses only the high dose and thereby reduces animal use by up to 40%.
  - If existing information suggests a substance might have ACD hazard potential and dose-response information is needed, consider testing in the multidose LLNA: DA.

Figure 1. LLNA: DA Test Method Protocol



Abbreviations: RLU = relative luminescence units; SI = stimulation index; SLS = sodium lauryl sulfate

Current Validation Status of the LLNA: DA

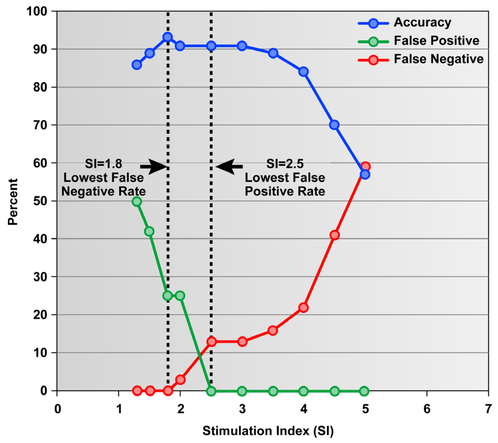
Accuracy

- LLNA: DA database of 44 substances
  - Idehara et al. 2008
  - Idehara, unpublished data
  - Omori et al. 2008 (interlaboratory validation study)
- Results compared to traditional LLNA data
- Stimulation index (SI) ≥ 1.8 produced optimal results based on no false negatives (**Figure 2**)
- The LLNA: DA correctly identified all 32 LLNA sensitizers (0% [0/32] false negatives) and 9/12 LLNA nonsensitizers
  - Accuracy = 93% (41/44)
  - False positive rate = 25% (3/12)
    - Chlorobenzene, hexane, and salicylic acid: all 1.8 < SI < 2.5
  - False negative rate = 0% (0/32)

Reliability

- A concordance analysis of sensitizer (10/14) and nonsensitizer (4/14) outcomes was conducted across two phases of an interlaboratory validation study.
  - Concordance was observed for 80% (8/10) of the sensitizer outcomes.
    - Two LLNA sensitizers, 3-aminophenol (1/3 SI < 1.8 and 2/3 SI ≥ 1.8) and nickel (II) sulfate hexahydrate (4/8 SI < 1.8 and 4/8 SI ≥ 1.8) produced discordant LLNA: DA test results.
  - Concordance was observed for 75% (3/4) of the nonsensitizer outcomes.
    - The discordant LLNA nonsensitizer was isopropanol (91% [10/11] concordance).

Figure 2. SI Decision Criteria Performance of the LLNA: DA Compared with the Traditional LLNA Using 44 Substances



Compared to traditional LLNA results, the lines show the change in performance characteristics for the LLNA: DA with the SI used to identify skin sensitizers. This analysis used LLNA results for 32 sensitizers and 12 nonsensitizers. For 14 substances with multiple LLNA: DA test results, the most prevalent outcome was used.

LLNA: DA Test Method Usefulness and Limitations

- The LLNA: DA can be used to identify potential skin sensitizers or nonsensitizers.
  - Use SI ≥ 1.8 to identify potential skin sensitizers.
- A slight potential for false positives with borderline weak positive responses (1.8 < SI < 2.5) exists.
  - Consider additional information such as dose-response relationship strength, statistical significance, evidence of systemic toxicity and/or excessive skin irritation together with SI values.
- The LLNA: DA might not be appropriate for testing certain classes of materials with properties that interfere with the assay. Consider if test substance might affect:
  - ATP levels (e.g., ATP inhibitors)
  - Accurate intracellular measurement of ATP levels (e.g., ATP-degrading enzymes or extracellular ATP in the lymph node)

LLNA Peer Review Panel Meetings

- Public meetings of an international independent scientific peer review panel were held at the Consumer Product Safety Commission in Bethesda, MD, on March 4-6, 2008, and at the National Institutes of Health in Bethesda, MD, on April 28-29, 2009.

Charge to the Peer Review Panel

- Review the draft Background Review Document (BRD) for errors and omissions
- Provide conclusions and recommendations on the current validation status of the LLNA: DA
- Does the information contained in the draft BRD support ICCVAM's draft test method recommendations?

Peer Review Panel Conclusions

- Concurred that the available data and test method performance supported the use of the LLNA: DA to identify substances as sensitizers and nonsensitizers, with certain limitations
- Recommended that before additional animal testing is conducted, consideration be given to the necessity for the substance to be tested for skin sensitization potential
- The complete LLNA Peer Review Panel Reports can be accessed at:
  - http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
  - http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2009.pdf

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International Acceptance of the LLNA: DA

- ICCVAM agreed with the OECD Expert Consultation Group that a single SI ≥ 1.8 to classify substances as skin sensitizers would avoid false negative and indeterminate results, which are not useful for regulatory purposes.
- OECD Test Guideline 442A Skin Sensitization: Local Lymph Node Assay: DA, which includes the SI ≥ 1.8 to classify substances as skin sensitizers, was adopted on July 22, 2010 (OECD 2010).
- OECD Test Guideline 442A can be accessed at http://www.oecd-ilibrary.org/
- International acceptance of the LLNA: DA is expected to result in broader use of LLNA tests.
  - Will further reduce and refine animal use for ACD hazard assessments on a global basis, while ensuring human safety
  - Will reduce costs and environmental hazards associated with the use of radioactive substances

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NIEHS National Institute of Environmental Health Sciences

NTP National Toxicology Program
U.S. Department of Health and Human Services